

## A FLUORESCENCE TECHNIQUE FOR THE DETECTION OF KNOBS IN THE MITOTIC CHROMOSOMES OF MAIZE

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### ABSTRACT

A fluorescence technique is described for the detection of knobs in the mitotic chromosomes of maize. The method possesses several advantages over conventional techniques based on pachytene analysis.

### UITTREKSEL

'N FLUORESSENSIE TEGNIEK VIR DIE OPSPORING VAN KNOPPE IN DIE MITOTIESE CHROMOSOME VAN MIELIES

'n Fluoressensie tegniek vir die opsporing van knoppe in die mitotiese chromosome van mielies word beskryf. Die metode het sekere voordele bo ander konvensionele tegnieke wat op pagiteen analise gebaseer is.

### INTRODUCTION

Conventional techniques for the identification of maize chromosomes have relied to a great extent on the recognition of "chromosome knobs", these being condensed regions of chromosome visible most readily during the pachytene stage of meiosis in the pollen mother cells. However, Vosa and De Aguiar (1972) demonstrated that the positions of the knobs may also be recognised as bands in mitotic chromosomes treated by the Giemsa staining technique of Vosa and Marchi (1972).

The present work describes a fluorescence technique, by means of which the positions of the knobs in mitotic chromosomes may be recognised with even greater clarity. Like the Giemsa technique, the method is a c-banding technique for heterochromatin, based on an initial denaturation-reannealing. However, this is succeeded in the present case not by Giemsa staining, but by treatment with a fluorochrome, namely the AT-binding, bibenzimidazole derivative "Hoechst 33258".

### MATERIAL AND METHODS

Actively growing root tips of the South African maize cultivar "Early Pearl" were pre-treated by immersion in saturated, aqueous  $\alpha$ -monobromonaphthalene for 4-5 hours, rinsed in deionised water, and fixed in 3:1 ethanol:glacial acetic acid

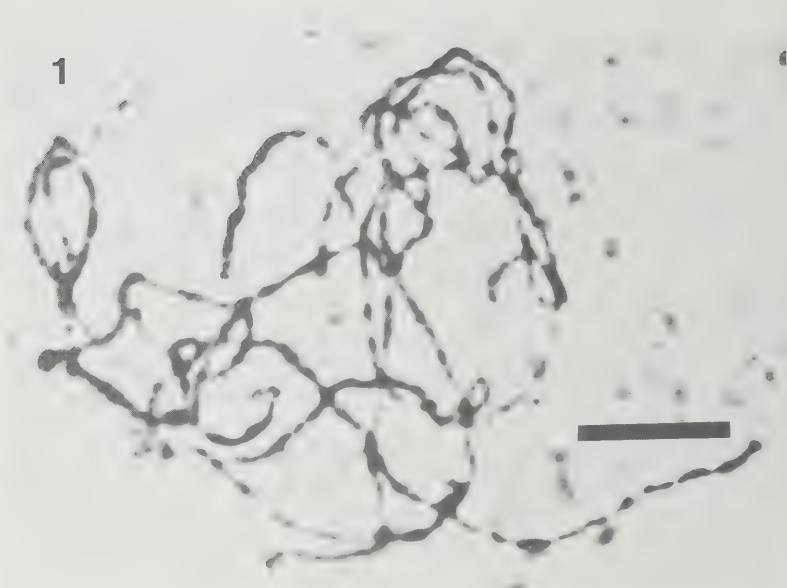


FIG. 1.

Chromosome Knobs in *Zea mays* Var. "Early Pearl".

Pachytene stage in pollen mother cell. Feulgen/Acetic orcein staining, phase contrast.

Bars represent 10  $\mu$

for 8–24 hours. They were then hydrolysed in 0,2N HCl at 60 °C for 2½ minutes, and squashed in 45 % acetic acid under an albumenised coverslip, the latter being floated off in absolute alcohol and air-dried. At this stage the preparations could be stored indefinitely at room temperature without appreciable deterioration.

Following this, the coverslips were immersed for 6 minutes in a saturated, aqueous solution of barium hydroxide at room temperature, rinsed in running deionised water, incubated in 2 X saline sodium citrate buffer at 60 °C for one hour, rinsed again in deionised water, and air-dried. The preparations were then stained for 5 minutes in a 0,02 % ethanolic solution of Hoechst 33258, rinsed in ethanol, air-dried, mounted in 50 % glycerol, and viewed using a Zeiss fluorescence microscope with exciter filter BG12 and barrier filters 50 plus 53. Photographs were taken using Kodak Tri-X Pan film.

If desired, the preparations could then be made permanent by Giemsa staining according to the method of Vosa and Marchi (1972). The procedure in this case was for the coverslips to be floated off in deionised water, stained for 1 hour in a 0,5 % solution of Giemsa buffered to pH 6,8, air-dried and mounted in Gurr's Depex.

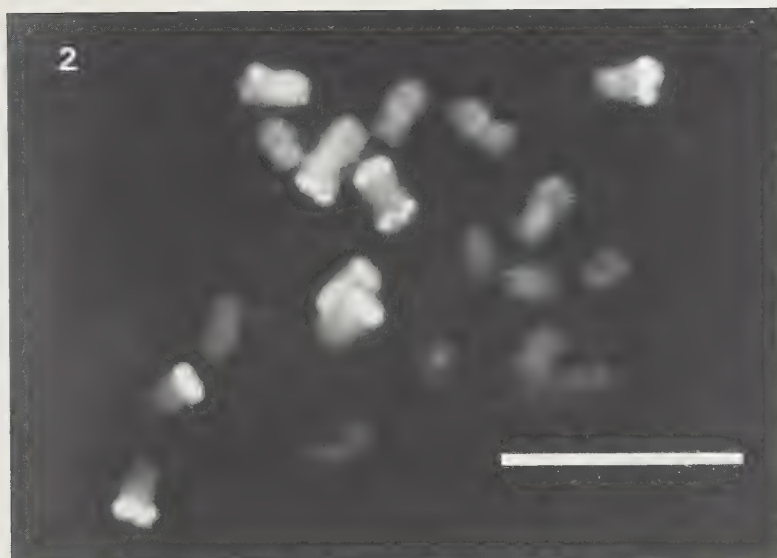


FIG. 2.

Chromosome Knobs in *Zea mays* Var. "Early Pearl".

Root tip metaphase, stained by fluorescence technique described in text.

Bars represent 10  $\mu$ .

## RESULTS AND DISCUSSION

The knob regions are revealed by the fluorescence technique as bands of intense fluorescence (Fig. 2), and correspond to those regions staining heavily with Giemsa (Fig. 3). Both the fluorescence and the Giemsa methods yield good results, though for the most part the fluorescence technique is the more consistent, being less sensitive to minor variations in the temperature of denaturation and the duration of staining.

Both methods possess many advantages over conventional techniques based on pachytene analysis. The latter frequently necessitate waiting considerable periods of time for meiotic material to become available, and resolution of the pachytene chromosomes themselves may be difficult due to their length, entanglement, and fusion of the knob regions. The degree of clumping of the chromosomes at pachytene is in fact itself under genetic control (Wellwood and Randolph, 1957).

In contrast, the fluorescence and Giemsa techniques, being based on mitotic chromosomes, remove almost all the above difficulties and greatly facilitate karyotype analysis.

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FIG. 3.

Chromosome Knobs in *Zea mays* Var. "Early Pearl".

The same cell as in Figure 2, stained by the Giemsa technique of Vosa and Marchi (1972).

Bars represent 10  $\mu$ .

## ACKNOWLEDGEMENT

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